Function of Column Length in Fast Chromatography

Application

Technical

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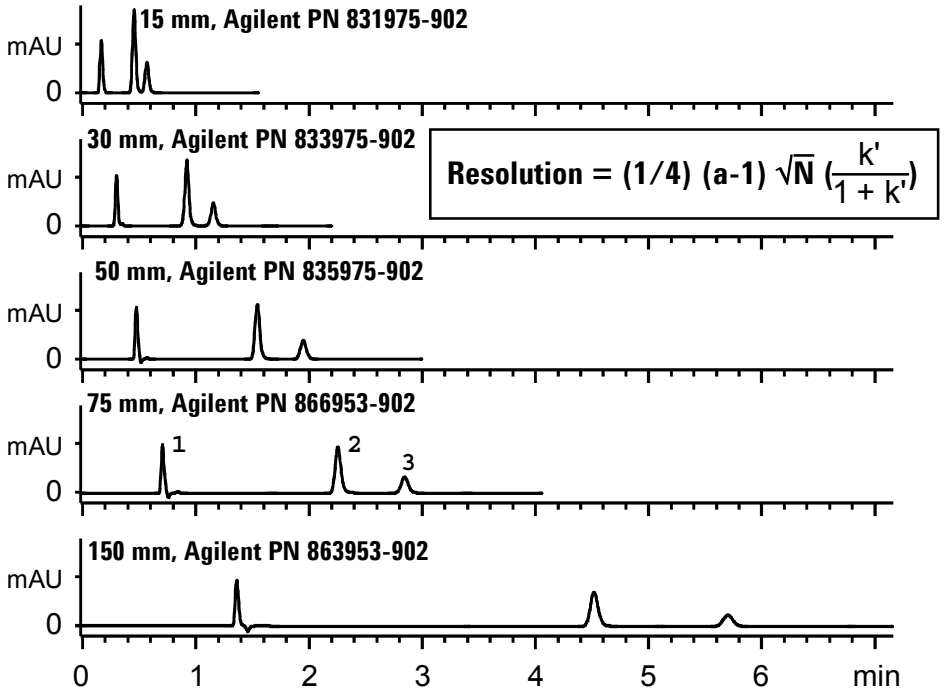
Today's fastest HPLC methods usually employ low-volume columns. Shortening column length does not alter k' or selectivity (a) obtained when using the longer-column method. Thus, converting to a faster method is as easy as installing a shorter column and adjusting some method parameters in the chromatography software, such as detector response time. Here, analgesics are analyzed on a series of Agilent ZORBAX SB-C18 Rapid-Resolution (3.5 µm) columns (4.6 x 150 mm, 75 mm, 50 mm, 30 mm, and 15 mm). Note as column length is reduced there is:

- reduction in analysis time
- increase in peak height
- tolerable loss of resolution

Differences in resolution are explained by the resolution equation. One can alter resolution by varying N, a , or k'. Here, k' and a are constant because mobile phase, stationary phase, and temperature are constant. Thus, resolution changes because of N. Factors that influence N are particle size, temperature, extra-column volume, and column length. In this case, all the factors are constant, except column length. To realize full potential of these columns, minimize extra-column effects.



$$\text{Resolution} = (1/4)\,(a\text{-}1)\,\sqrt{N}\,\left(\frac{k'}{1+k'}\right)$$

Highlights

- *Low-volume columns are valuable for high-speed analyses, decreasing solvent consumption, and enhancing sensitivity for mass-limited samples commonly used for LC/MS applications.*
- *Low-volume cartridge columns are as rugged and reproducibile as the larger, traditional end-fitting columns.*
- *Switching from a 150-mm to a 15-mm column reduces analysis time and solvent use ten-fold.*

Conditions:
LC: Agilent 1100
Column: ZORBAX SB-C18 (3.5 µm)
Mobile Phase: ACN : H_2O, 1% formic acid (32:68); pH 2.3
UV: 254 nm; Flow: 1.0 mL / min.; 30°C
Inj. Vol.: 5 µL

1. Uracil
2. Acetanilide
3. Acetosalicylic acid

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